PATENT
Client-Matter No.: 66667-011
(P-ZA 3519)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Maurizio Zanetti	)	Group Art Unit: 1632
Serial No.: 09/300,959	)	Examiner: A. Wehbe
Filed: April 27, 1999	)	Confirmation No.: 5037
For: SOMATIC TRANSGENE IMMUNIZATION AND RELATED METHODS	)	

Commissioner for Patents
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DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Maurizio Zanetti, declare as follows:

1) I am the Maurizio Zanetti who is named as an inventor on the above-identified patent application.

2) I understand that the claims of the subject application stand rejected, in part, as allegedly lacking enablement.

3) With regard to administration to lymphoid tissues other than spleen, I believe that lymphoid tissues share common properties that allow nucleic acids administered to a lymphoid tissue to stimulate an immune response similar to that demonstrated for administration to spleen. For example, lymphoid tissues share the common property of relatively high

populations of B cells. In particular, peripheral blood contains about 10-15% B lymphocytes, spleen contains about 40-50% B lymphocytes, lymph nodes contain about 20-25% B lymphocytes, and Payer's patches contain about 60-70% B lymphocytes. Given that lymphoid tissues contain a high population of B lymphocytes, I believe that a nucleic acid administered to a lymphoid tissue will be in proximity to B cells sufficient for uptake of the nucleic acid in B cells an expression of an encoded polypeptide from a B cell expression element.

4) With regard to targeting a nucleic acid molecule to a B cell *ex vivo*, experiments have been performed to test *ex vivo* transgenesis of B lymphocytes in human blood. Briefly, normal peripheral blood lymphocytes (PBL) were isolated and incubated with plasmid vector containing an epitope of the MART-1 melanoma-associated antigen inserted in the CDR3 of the pNeoγ1 vector, with enhanced green fluorescent protein (EGFP) inserted at the C terminus of the γ1 constant region, under the control of a B cell expression element. EGFP functions as a readily detectable marker for expression from the B cell expression element. To assess whether B lymphocytes are the target cells in PBL, EGFP-positive cells in PBL were stained with phycoerythrin (PC)-conjugated murine monoclonal antibody against CD19, a marker of human B lymphocytes. 85-90% of detectable EGFP-positive cells were also CD19-positive (see Exhibit A, panels a and b). Thus, essentially only B lymphocytes undergo transgenesis and express the EGFP marker. Since PBL contain 10-15% B lymphocytes, the average efficiency of spontaneous transgenesis in the B lymphocyte population is estimated to be

approximately 10%. The B lymphocytes in PBL are divided into two general categories, naive and memory, which in humans are distinguished by the expression of membrane CD27. Transgenic lymphocytes that were EGFP-positive were found to be IgD-positive/CD27-negative cells (Exhibit A, panels c and d). This indicates that naive mature B lymphocytes are the target population.

5) Spontaneous *ex vivo* transgenesis of B lymphocytes has been performed reproducibly in various species. About 0.5% of lymphocytes underwent transgenesis in mouse, about 2% in macaque and about 1% in human. Since about 10-15% of human lymphocytes are B lymphocytes, about 10% of human B cells undergo transgenesis. Therefore, a variety of species can undergo *ex vivo* transgenesis of B lymphocytes.

6) In conclusion, a nucleic acid containing a B cell expression element can be targeted to a B cell both *in vivo* and *ex vivo* and results in expression of an encoded heterologous polypeptide in B cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date: 11/21/2003 By: _____

Maurizio Zanetti